2744-Pos Board B514
Ion-Channel Coupled Receptors: New Tools for the Study of Receptors and Channels
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Ligand-gated ion channels combine within a single polypeptide chain a binding site for a specific signalling molecule and a selective ion-conducting pore, allosterically linked so that ligand binding controls pore opening, thus allowing fast chemical-to-electrical signal transduction as required in synapses.
Ion Channel-Coupled Receptors (ICCRs) are artificial proteins comprised of a G protein-coupled receptor fused to an ion channel, engineered to create a physical coupling between ligand binding and channel gating. The ICCR concept was previously validated with fusion proteins between the K^+ channel Kir6.2 and either muscarinic M_2, or dopaminergic D_2 unmodified receptors (Moreau *et al.*, *Nature Nanotech.* **3:**620-625, 2008). Here, we extend the concept to the β_2-adrenergic and the opsin receptors.
These receptors raised new challenges as they are significantly different from M_2 and D_2 receptors in terms of structure (poor sequence homology, much longer cytoplasmic C-terminal), function (coupled to distinct G-proteins, distinct effectors), and surface expression (extremely low in some cases in our *Xenopus* oocyte heterologous expression system). We show here how surface expression can be enhanced by co-expression of an accessory Kir6.2-binding protein, the first transmembrane domain of the sulfonylurea receptor SUR, and how receptor-channel coupling can be achieved by modifications of the receptors that preserve their ligand-binding properties. The resulting β_2 and opsin ICCRs constitute blueprints for innovative tools to study the molecular mechanics of receptors and channels and could find applications not only in diagnostics and drug screening, but also in synthetic biology as adrenergic-gated and light-gated K^+ channels.

2745-Pos Board B515
SUR1 L0 and Kir6.2 M0 can Partner in KATP Gating: Evidences from Diabetogenic Mutations
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Neonatal diabetes (ND) mutations in different domains of either KATP subunit overactivate KATP via different mechanisms. Mutations in the ABC core of SUR1 augment its Mg-nucleotide-dependent stimulation of Kir6.2. Mutations at the putative ATP site of Kir6.2 supposedly compromise the inhibitory nucleotide binding. Mutations at the putative gate reportedly stabilize its open state. Many ND mutations map to the putative L0 helix preceding the SUR1 core and to the M0 ('slide') helix of Kir6.2. We discovered that L0 controls spontaneous bursting and introduced a model in which L0 and M0 partner in KATP gating (Babenko & Bryan 2003). The model predicted that ND mutations in either amphipathic helix at the membrane-cytosol interface hyperactivate KATP by altering its intrinsic gating kinetics. Here we tested the idea. We compared the effects of severe ND mutations in the middle of either interface helix on spontaneous single-channel kinetics, ATP-inhibition, and Mg-nucleotide stimulation of KATP. We found that each of these mutations decreases the rate of burst termination while increasing the rate of burst initiation, thereby reducing the availability of the closed state with the lowest Kd for inhibitory ligands. This mechanism attenuates ATP inhibition but not Mg-nucleotide stimulation, thereby hyperactivating KATP in vivo, and uncouples sulfonylurea binding from Kir6.2 closure. Thus, in support of our model, perturbing either interface helix disrupts SUR1/Kir6.2 inhibitory coupling. Affinity photolabeling with I-azidoglibenclamide indicated that none of the ND mutations altered the proximity of L0 to M0. But each of these mutations changed the helical hydrophobic moment, implying that rotation of either helix along its axis disrupts L0/M0 interactions. We propose that the two interface helices have been optimized to interact and slide together to stabilize the long-lived closed state of SUR1/Kir6.2 complex.

2746-Pos Board B516
Molecular Modelling and Experimental Analysis of the ATP Sensitivity of Cloned Smooth Muscle KATP Channels
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ATP-sensitive K^+ (K_{ATP}) channels, comprised of pore-forming $K_{ir}6.x$ and regulatory SURx subunits, play important roles in many cellular functions. Vascular ($K_{ir}6.1$/SUR2B) and non-vascular ($K_{ir}6.2$/SUR2B) smooth muscle K_{ATP} channels regulate the resting membrane potential in smooth muscle cells and modulate the muscle tone. In spite of the fact that $K_{ir}6.1$ and $K_{ir}6.2$ share ~70% sequence identity, their sensitivity to ATP inhibition differs by more than 30-fold with IC_{50} values of 3.1 ± 1.3mM (n=7) and $101 \pm 22\mu$M (n=17), respectively. We investigated the molecular mechanisms that underlie this differential response. All (17) $K_{ir}6.2$ residues putatively involved in binding of ATP are conserved in $K_{ir}6.1$ with the exception of K39 and K185. We constructed a homology model of $K_{ir}6.2$ and carried out computational docking of ATP to determine the conformation of bound ATP in its putative binding site. The model was validated by assessing the binding free-energy difference of ATP for wild-type ($K_{ir}6.2$/SUR2B) and a range of $K_{ir}6.2$ ATP-binding site mutants for which the affinity for ATP was previously experimentally determined. The model predicted that substitution of K39 and K185 on $K_{ir}6.2$ with the equivalent residues on $K_{ir}6.1$ (K39S/K185R) did not affect the ATP affinity. Patch-clamp experiments confirmed that there was no difference in the ATP sensitivity between these channels. Furthermore, the open probability (P_o) of $K_{ir}6.1$/SUR2B, $K_{ir}6.2$/SUR2B and $K_{ir}6.2$-K39S-K185R/SUR2B were identical (0.41 ± 0.05 (n=7), 0.38 ± 0.06 (n=7) and 0.45 ± 0.02 (n=8), respectively), indicating that their different ATP sensitivity is not caused by a change in channel gating. In conclusion, the difference in ATP sensitivity between $K_{ir}6.1$ and $K_{ir}6.2$ is not caused by a difference in the ATP binding site meaning other regions of the channel must be involved.
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Cardiac Electrophysiology II

2747-Pos Board B517
Patient-Specific Inducible Pluripotent Stem Cells Reveal Mechanism of Personalized Therapy for an Inherited Cardiac Arrhythmia
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We report a study using inducible pluripotent stem cells (iPSCs) designed to unravel the mechanistic basis for resistance to previously prescribed gene-specific therapy for a patient carrying a Long QT Syndrome variant 3 (LQT3) mutation. The previous therapy, treatment with the Na^+ channel blocker mexiletine, had been based on previous heterologous expression studies. In contrast to previous predictions arrhythmias in the proband have not been controlled by mexiletine, which has had a limited therapeutic dose range because of increased clinical risk found at high doses. The proband harbors a *de novoSCN5A* mutation (F1473C) and is heterozyous in *KCNH2* (K897/T897). Both parents lack the *SCNA5* mutation and each is homozygous for either T897 or K897 *KCNH2*. Cardiomyocytes (hiPSC-CMs) were derived from hiPSCs produced by lentiviral reprograming of skin fibroblasts. Whole-cell patch clamp was performed on single hiPSC-CMs from each family member and confirmed dysfuntional Na^+ channel inactivation in cells from the proband but neither parent which was corrected by mexiletine (50 μM). However this same mexiletine concentration was found to also have sginficant off-target block of L-type calcium and I_{Kr} potassium channels, which may explain the patient's suboptimal response to the therapeutic regimen and limited useful therapeutic dose range of the drug. Instead of raising drug concentration we show that increased heart rate, which in the patient is controlled by an implantable cardioverter-defibrillator (ICD), effetively and markedly inhibits dysfunctional Na^+ channel activity in these cells and has markedly suppressed arrhythmic events in the proband. These results demonstrate the power and utility of iPSCs in detecting mechanisms of disease and optimizing its treatment in patient-specific manner.

2748-Pos Board B518
Alternating Cycle Lengths Increases Dispersion of Action Potential Durations (APD) in Transgenic Rabbit Model of Long QT Syndrome Type 2
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Long QT syndrome Type 2 (LQT2) is a congenital disease with abnormal prolongation of APD and sudden cardiac death (SCD) due to polymorphic ventricular tachycardia (pVT). These arrhythmias are often preceded by a characteristic initiation mode of short-long-short alternating cycle lengths (CL) in surface electrocardiograms. We hypothesized that alternating CLs in LQT2 cause increased APD dispersion, which may promote reentry formation.